



Neutrophil infiltration is implicated in the sustained thermal hyperalgesic response evoked by allergen provocation in actively sensitized rats

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Abstract

It has been proposed that allergen provocation induces hyperalgesia but the involvement of immunoglobulin E and leukocytes remains poorly understood. Here, we have compared the profile of allergen-evoked thermal hyperalgesic response in both passively and actively sensitized rats, and investigated the role of leukocytes in allergen-evoked nociception. Wistar rats were passively sensitized with an intraplantar injection of immunoglobulin E anti-dinitrophenylated bovine serum albumin monoclonal antibody (0.5 µg/paw), and challenged with dinitrophenylated bovine serum albumin (0.5 µg/paw) 24 h later. Alternatively, the animals were actively sensitized with a mixture of Al(OH)₃ and ovalbumin and challenged intraplantarly with ovalbumin (12 µg/paw) 14 days later. We found that the thermal hyperalgesic responses set in very rapidly and with comparable intensity in both passively and actively sensitized rats. However, while in the former group the response was shorter, peaking within 1 h and reducing thereafter, a marked plateau was observed from 1 to 6 h post-challenge in the latter group. Actively sensitized rats also had higher neutrophil influx in the plantar tissue, as attested by both myeloperoxidase activity and histological analysis. Treatment of actively sensitized rats with either fucoidin (10 mg/kg, i.v) or anti-rat neutrophil antiserum (i.p.) reduced neutrophil accumulation and the late hyperalgesic response noted from 3 to 6 h post-challenge. Thus, we conclude that though immunoglobulin E-mediated mechanisms can cause thermal hyperalgesia, components of the cellular immune reaction are crucial in order to amplify and sustain the immediate hyperalgesic response triggered by allergen, in a process dependent on neutrophil recruitment.

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1. Introduction

Most of the symptoms of acute-phase allergic reactions result from the rapid release of inflammatory mediators following the allergen-evoked cross-linking of immunoglobulin E (IgE) high-affinity receptors expressed on the surface of mast cells (Corry and

Kheradmand, 2006). It is well established that exposure of the skin, nose, airways or intestinal tract of atopic individuals to allergen challenge leads to inflammation within minutes, causing both tissue damage and nerve injury (Williams and Galli, 2000). One of the cardinal features of the inflammatory state is that normally innocuous stimuli produce pain (Kidd and Urban, 2001), but there are doubts concerning the putative implication of pain mechanisms in allergic conditions.

Little information is available on allergen-evoked nociception. According to Van Arm, allergic lesions

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including urticarial wheals and passive cutaneous anaphylaxis are marked by the production of itch instead of pain (Van Arman et al., 1968). Nevertheless, it has been proposed that itching could indeed be a distinct quality of nociception, also resulting from the stimulation of nociceptors in the superficial layers of the skin (Heyer and Hornstein, 1999). It is noteworthy that patients with allergies show a higher psychoemotional response to pain provocations when compared with patients without the accompanying pathology (Stosh et al., 1991).

The ability of allergen challenge to induce hyperalgesia in sensitized rats has been investigated in recent studies. Findings show that a thermal hyperalgesic state is present in the area of tissue damage created by allergen challenge in IgE passively sensitized rats. The phenomenon appears to be independent of the concomitant oedematogenic response and critically mediated by the synergistic interaction of vasoactive amines and bradykinin (Lavich et al., 2003). Studies revealed that allergen challenge caused a dose- and time-dependent mechanical hypersensitivity, which peaked within 3 h returning to the baseline levels within 24 h, and that TNF α , prostaglandins, sympathetic amines and leukotriene (LT) B $_4$ are crucially involved in allergic nociception in immunized rats (Cunha et al., 2003).

Interactions between nerves and inflammatory cells lead to a number of important physiological responses, including pain, during inflammatory reactions (Brown, 1991; Fu and Gordon, 1997). It is well established that immune cells release various algogenic mediators, leading to a state of priming and stimulation of nociceptors (Levine et al., 1986; Taiwo and Levine, 1990, 1992). We and others have reported that the allergen challenge induced intense exudatory responses in rats and mice passively sensitized with IgE, which were comparable to those induced in immunized rodents, but failed to mimic the magnitude of leukocyte recruitment (Lima et al., 1997; Kaneko et al., 2000; Calheiros et al., 2001). In this study, we aimed at investigating the putative implication of the IgE-mediated system in triggering nociception as well as the potential involvement of leukocytes in processing allergic hyperalgesia. Our findings indicate that neutrophil infiltration into the focus of the lesion distinguishes thermal hyperalgesic responses evoked by allergen provocation in actively as compared to IgE passively sensitized rats.

2. Material and methods

2.1. Animals

All experiments were performed in male Wistar rats, weighing between 180 and 220 g. The animals were obtained from the breeding colony of the Oswaldo Cruz Foundation, Rio de Janeiro, Brazil, housed in groups of five and maintained on a 12-h light/dark cycle, with water and food ad libitum until

use. The Ethics Committee for Care and Use of Laboratory Animals of Oswaldo Cruz Foundation approved the experimental protocols used in this study (License no. 0085-02).

2.2. Allergy sensitization and hindpaw challenges

2.2.1. IgE sensitization

In the group of IgE sensitized rats, murine IgE anti-dinitrophenylated (DNP-BSA) MAbs (0.5 μ g/paw, 100 μ l) and 0.9% NaCl (saline) (100 μ l) were intraplantarly injected into the left and right hindpaws, respectively. In another group (sham-sensitized rats), 100 μ l of saline was injected into both left and right hindpaws. Sensitized and sham-sensitized left paws were then challenged with an intraplantar injection of allergen (DNP-BSA, 0.5 μ g/paw) 24 h later, whereas contralateral paws were injected with vehicle (saline, 100 μ l).

2.2.2. Active sensitization

In another experimental set, rats were immunized by a subcutaneous injection of ovalbumin (50 μ g/0.2 ml) dissolved in an emulsion containing 5 mg of Al(OH) $_3$, and the volume was completed with sterile saline. After 14 days the animals were challenged with ovalbumin (12 μ g/paw) in the left paw, while the right paw was injected with vehicle (sterile saline, 100 μ l). Two negative control groups were used: (i) first, there were sham-sensitized rats subjected to the same scheme of plantar provocations, in which sterile saline solution replaced ovalbumin in the Al(OH) $_3$ suspension; (ii) second, there were sham-challenged rats in which challenge with ovalbumin was replaced by challenge with saline.

2.3. Algesimetric assay

Thermal hyperalgesic response was evaluated as previously described (Lavich et al., 2005). Briefly, animals were individually placed on a hot plate (Eddy's Hot Plate) with the temperature adjusted to 51 $^{\circ}$ C and the withdrawal response latency of each hind paw determined at 15, 60, 180 and 360 min after allergen challenge. The heat source was maintained at constant intensity, which caused a stable withdrawal latency of approximately 8–10 s in sham-challenged paws. The animals were only tested in one series of measurements, and the typical responses were hind paw shaking and/or lifting. The latency to the response was manually recorded with a chronometer. The experiments were performed in a sound-attenuated and air-conditioned (20–22 $^{\circ}$ C) room. Hyperalgesia to heat was defined as a decrease in withdrawal latency and calculated as follows: Δ paw withdrawal latency (s) = right paw withdrawal latency – left paw withdrawal latency.

2.4. Measurement of myeloperoxidase activity

Neutrophil recruitment into the rat paw was indirectly assessed by measurement of neutrophil myeloperoxidase (MPO) activity. Animals were killed in a CO $_2$ atmosphere and the subcutaneous tissue of the paws was removed, weighed and homogenized at 5% in a PBS solution containing 5 mM EDTA and 0.5% hexadecyltrimethyl ammonium bromide (HTAB). Samples were then centrifuged at 9000g for 15 min

at 4 °C and the supernatant evaluated for myeloperoxidase activity. Enzymatic reaction was assessed as previously described (Bradley et al., 1982). The following reagents were added to wells of 96-well microtiter plate: 50 μ l of PBS solution containing EDTA (5 mM) and HTAB (0.5%), 50 μ l of the supernatant, 50 μ l *o*-dianisidine (0.68 mg/ml in distilled water) and 50 μ l of freshly prepared 0.003% hydrogen peroxide to start the reaction. Absorbance was measured at 460 nm in plate reading (Versa Max – Molecular Device, Sunnyvale, CA, USA) and results expressed in optical density (OD).

2.5. Histopathology and neutrophil enumeration

After killing the animals in a CO₂ atmosphere, the subcutaneous tissue of the paws was removed and fixed in Carson-Millonig phosphate-buffered 4% formaldehyde fixative (Herre-ra, 1992). Routine histological techniques were used to paraffin-embed the entire tissue, which was sectioned at 5 μ m and stained with H&E. The tissue sections were examined with an Olympus B201 microscope to check the presence of inflammatory infiltrates at 60, 180 and 360 min after allergen challenge. To assess the number of neutrophils in tissue, each section was evaluated at 1000 magnification on optical microscope and the cells were counted in fifty fields. The mean value of the fifty areas was taken as representative of the whole section.

2.6. Treatments

Sensitized animals were treated with the selectin inhibitor fucoidin (10 mg/kg) administered by intravenous injection 15 min before allergen challenge. Fucoidin was dissolved in sterile saline. Neutrophil depletion was performed by administration of a rabbit anti-rat neutrophil antiserum (2 ml/kg, i.p.), 18 h prior to the allergen challenge (Bennett et al., 1998). In control groups, animals were treated with sterile saline and rabbit normal serum, respectively.

2.7. Material

IgE anti-DNP-BSA was kindly donated by Dr. Anne Provoust Danon (Institut Pasteur, França). DNP-BSA, OVA, fucoidin, EDTA, hexadecyltrimethyl ammonium bromide (HTAB) and *o*-dianisidine were purchased from Sigma Chemical Company (St. Louis, MO, USA). Anti-neutrophil antiserum was purchased from Accurate Chemicals, USA.

2.8. Statistical analysis

All results were given as means $\pm$ standard error of the mean and statistically analyzed by one-way analysis of variance (ANOVA), followed by Newman–Keuls Student's *t*-test. *P* values of 0.05 or less were considered significant.

3. Results

3.1. Kinetics of the hyperalgesic response following allergen provocation of actively and IgE passively sensitized rats

Kinetics of the development of allergen-evoked thermal hyperalgesia is illustrated in Fig. 1. The thermal

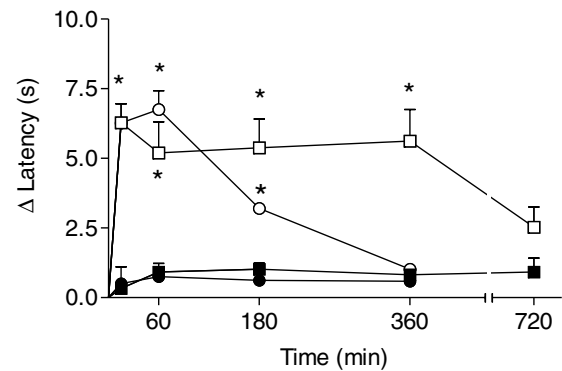


Fig. 1. Kinetics of thermal hyperalgesia induced by ovalbumin (12 μ g/paw) and DNP-BSA (0.5 μ g/cavity) in actively (unfilled square) and IgE passively sensitized rats (unfilled circle), respectively. In the negative control groups, ovalbumin and DNP-BSA were injected intraplantarly in rats sensitized with a mixture of Al(OH)₃ + saline (filled square) and saline (filled circle) (sham sensitized animals), respectively. Results are expressed as means $\pm$ SEM from at least six animals. **P* < 0.05 as compared to the respective sham sensitized group.

response set in very rapidly peaking from 15 to 60 min after antigen provocation both in passively and actively sensitized rats. However, the duration of allergen-evoked hyperalgesia under such conditions was clearly distinct. While in actively sensitized rats the hyperalgesic response was kept almost intact for at least 360 min post-challenge, remaining still elevated for at least 720 min, it reduced within 180 min and became unapparent within 360 min in IgE passively sensitized rats.

3.2. Extent and time-course of leukocyte infiltration

As compared to saline-injected paws (Fig. 2D), a mild leukocyte infiltration was noted 60 min after allergen provocation both in IgE passively sensitized rats (Fig. 2A) and in actively sensitized rats (Fig. 2E). As shown in Figs. 2F and G, there was a progressive increase in the leukocyte numbers at 180 and 360 min after allergen challenge in actively sensitized rats. When the histological analysis was performed under higher magnification (with the aid of oil immersion lens), we observed that the leukocyte infiltrate was marked by the presence of mononuclear cells and neutrophils (Fig. 2H). In addition, the analyses revealed that the leukocyte infiltration noted in passively sensitized rats appeared significantly less intense (Figs. 2B and C), compared with that infiltration noted in actively sensitized animals at 180 and 360 min post-challenge.

We have also measured neutrophil infiltration into the plantar tissue by measurement of myeloperoxidase (MPO). The results in Fig. 3 show that, compared to what was observed in sham-challenged control animals, the MPO activity in paw extracts of actively sensitized rats was 10- and 11-fold increased at 180 and 360 min

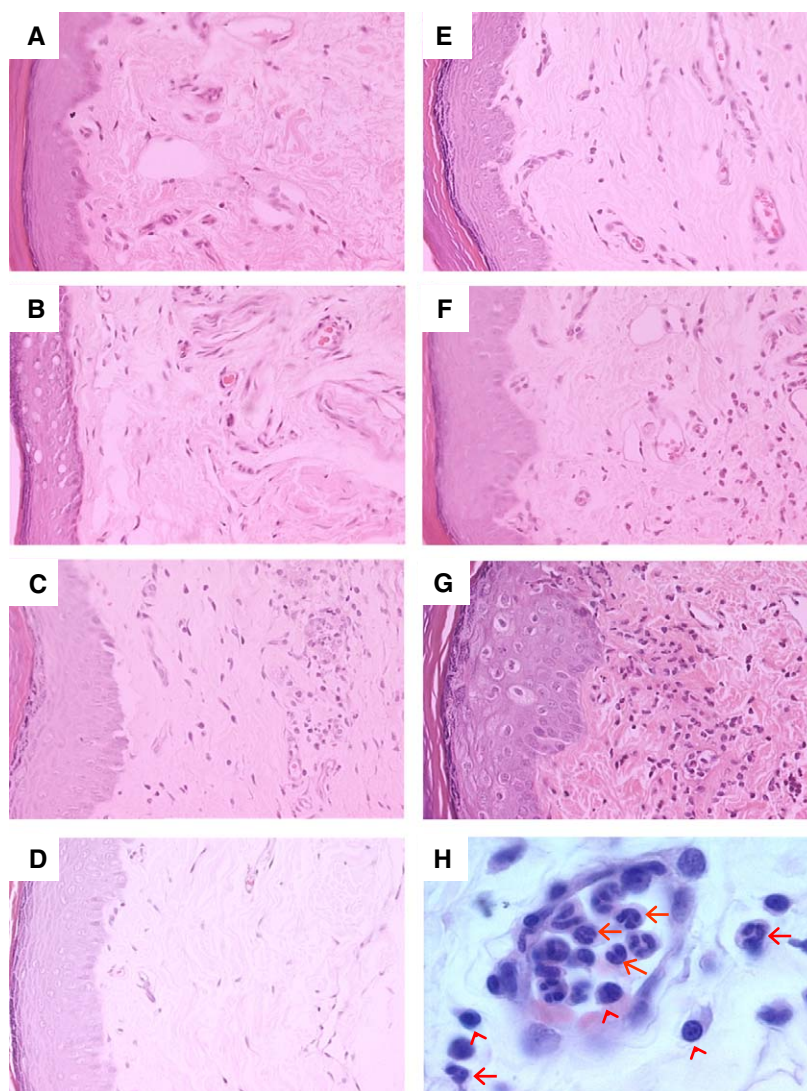


Fig. 2. Histologic analysis of plantar sections from IgE passively sensitized rats (A–C) and actively sensitized rats (E–H) challenged with DNP-BSA and ovalbumin, respectively. In time-points ranging from 60 to 180 min following antigen challenge, plantar skin tissues were removed and fixed with formaldehyde overnight. Representative 5- μ m sections of hind paw skin tissue were stained with hematoxylin and eosin and examined by means of light microscopy. (A, B and C) Representative plantar skin sections (200 $\times$) from IgE passively sensitized rats 60, 180 and 360 min after DNP-BSA provocation, respectively. (E, F and G) Representative plantar skin sections (200 $\times$) from actively sensitized rats 60, 180 and 360 min after ovalbumin provocation, respectively. (D) A representative plantar skin section (200 $\times$) from an IgE passively sensitized rat challenged with saline 360 min post-challenge. (H) A representative plantar skin section (analysis under oil immersion, 1000 $\times$) from an actively sensitized rat 360 min after ovalbumin challenge, in which perivascular and intravascular leukocyte accumulations are evident, mainly neutrophils (arrows) and mononuclear cells (arrow heads).

after allergen challenge, respectively. Similar experiments performed in IgE passively sensitized rats revealed increases of no more than 5-fold for the plantar MPO activity at the same time-points.

3.3. Effect of neutrophil depletion on allergen-evoked sustained hyperalgesia

In attempting to evaluate the putative role of neutrophils in allergen-evoked sustained hyperalgesia, experiments were carried out in which circulating neutrophils were depleted with a rabbit anti-rat neutrophil antise-

rum. The efficacy of the anti-neutrophil antiserum in actively sensitized rats is shown in Fig. 4. Treatment with the serum led to marked reduction in the number of neutrophils in the focus of the lesion, as attested by measurement of MPO activity in paw extract samples 360 min post-challenge (Fig. 4A). By comparison, treatment with the normal serum had no effect on allergen-evoked neutrophil recruitment as shown in the same figures. Of note, prior treatment of rats with the rabbit anti-rat neutrophil antiserum clearly inhibited allergen-evoked thermal hyperalgesia noted 180 and 360 min after allergen provocation as shown in Fig. 4B.

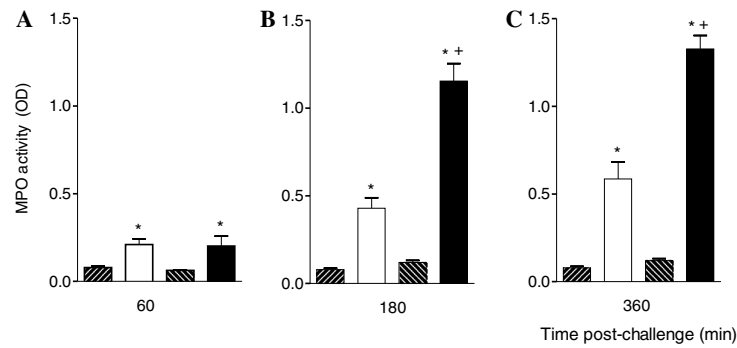


Fig. 3. MPO activity of extracts from allergen challenged rat hind paws, under conditions of active and passive IgE sensitization. MPO activity is shown for samples taken 60 min (A), 180 min (B), and 360 min (C) after either intraplantar injection of saline (left hatched and right hatched columns for active and passive sensitization, respectively) or allergen (open and closed columns for passive and active sensitization, respectively). Values are means $\pm$ SEM from at least six animals. * $P < 0.05$ as compared to the respective control (saline) injected rats. ** $P < 0.05$ as compared to the respective IgE passively sensitized rats.

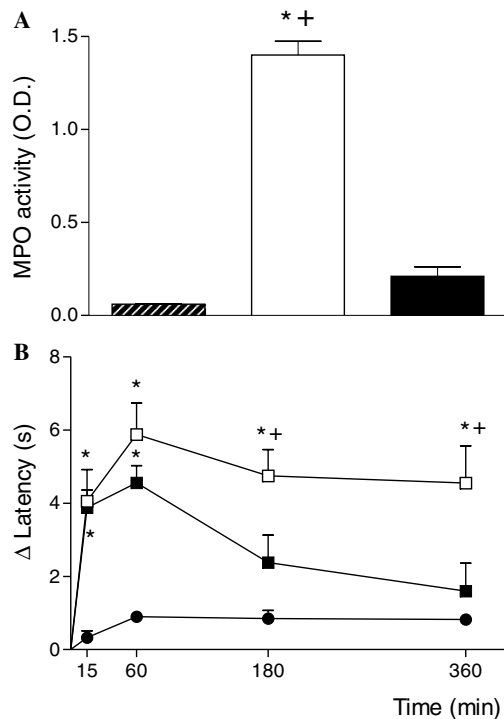


Fig. 4. (A) Effect of treatment with rabbit anti-rat neutrophil antiserum (closed column) or irrelevant antiserum (open column) on MPO activity of rat hind paw extracts from actively sensitized rats 360 min post-allergen challenge. Left hatched column represents values from actively sensitized rats challenged with saline. (B) Effect of treatment with rabbit anti-rat neutrophil antiserum (filled square) or irrelevant antiserum (unfilled square) on allergen-evoked hyperalgesia in actively sensitized rats. Negative control group was constituted by actively sensitized rats challenged with saline (filled circles). Values are means $\pm$ SEM from at least six animals. * $P < 0.05$ as compared to saline stimulated rats. ** $P < 0.05$ as compared to rats treated with rabbit anti-rat neutrophil antiserum.

3.4. Effect of fucoidin on allergen-evoked neutrophil recruitment and hyperalgesia

Experiments were also carried out in which the animals were treated intravenously with the selectin inhib-

itor fucoidin (10 mg/kg) in order to investigate whether blockade of neutrophil transendothelial migration would bring consequence to allergen-evoked thermal hyperalgesia. Histological analysis revealed that fucoidin significantly inhibited neutrophil influx into the plantar area 360 min following provocation of actively sensitized rats with ovalbumin as revealed by the enumeration of these cells in the focus of lesion (Fig. 5A). Furthermore, measurements of MPO activity in plantar extracts confirmed that the fucoidin treatment inhibited the neutrophil recruitment at 180 and 360 min post-challenge (Fig. 5B). Similar to what was observed following antiserum-induced neutrophil depletion, the fucoidin-induced blockade of neutrophil infiltration did not alter the allergen-evoked thermal hyperalgesia noted within 60 min, but significantly reduced the phenomenon at 180 and 360 min after allergen challenge (Fig. 5C).

3.5. Evaluation of the putative role of neutrophils in allergen-evoked hyperalgesia under conditions of IgE passive sensitization

As illustrated in Fig. 6, differently from to what was seen in the case of actively sensitized rats, those subjected to IgE passive sensitization showed a profile of allergen-evoked hyperalgesia clearly resistant to the depletion of circulating neutrophils or blockade of tissue neutrophil accumulation, as demonstrated by the anti-neutrophil antiserum and fucoidin treatments, respectively.

4. Discussion

Although the role of IgE in the immediate phase of the allergic reaction is well established (Poole and Rosenwasser, 2005), the potential involvement of this immunoglobulin in the mechanism of tissue cellular recruitment and nociception remains poorly understood. The principal result of this study is that the state

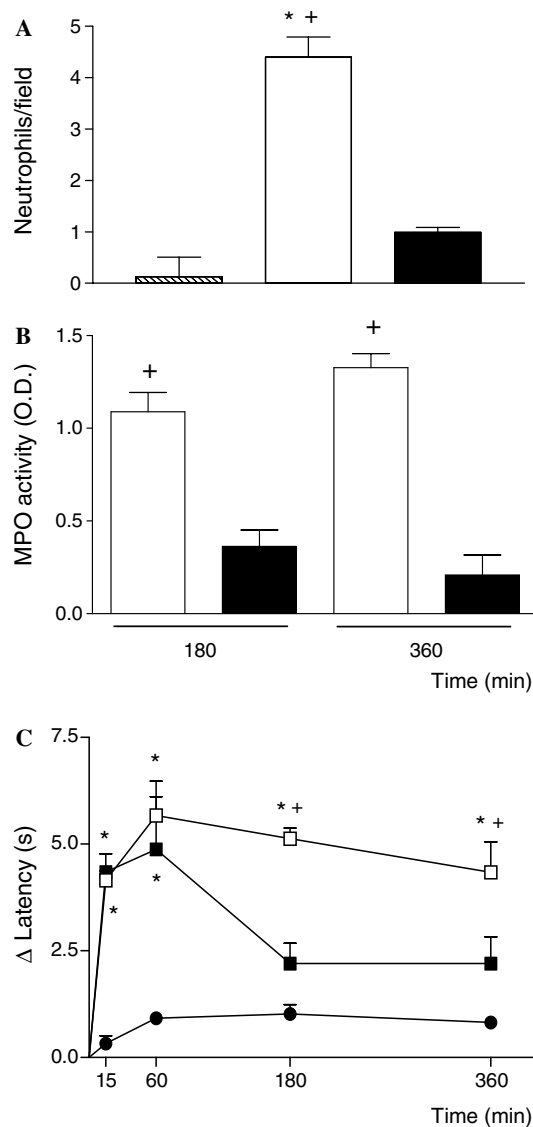


Fig. 5. (A) Neutrophil counts in plantar skin sections obtained 360 min after ovalbumin provocation in actively sensitized rats pretreated with fucoidin (10 mg/kg, i.v.) (closed column) or saline (i.v.) (open column). The negative control group consisted of actively sensitized rats injected with saline (hatched column). (B) Effect of fucoidin (10 mg/kg, i.v.) on MPO activity measured in plantar extracts from actively sensitized rats within 180 and 360 min post-challenge (closed columns) as compared to sensitized and ovalbumin challenged rats pretreated intravenously with saline (open columns). (C) Effect of treatment with fucoidin (10 mg/kg, i.v.) (filled squares) or vehicle (unfilled squares) on allergen-evoked hyperalgesia in actively sensitized rats. The negative control group consisted of actively sensitized rats challenged with saline (filled circles). Values are shown as means + SEM from at least six animals. $^+P < 0.05$ as compared to fucoidin-treated animals. $^*P < 0.05$ as compared to the negative control group.

of plantar thermal hyperalgesia created by allergen provocation lasts longer in actively sensitized rats when compared to that noted in IgE passively sensitized rats. Of note, significantly higher levels of neutrophils were attracted into the focus of allergen-evoked inflammation

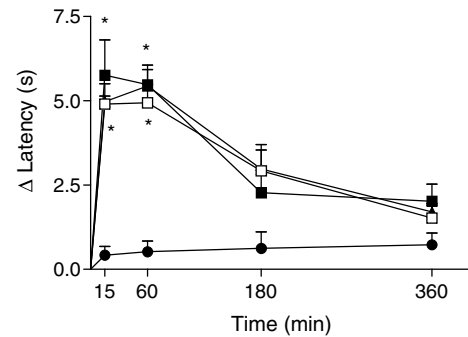


Fig. 6. Effect of treatment with rabbit anti-rat neutrophil antiserum (filled square), irrelevant antiserum (unfilled squares) and fucoidin (10 mg/kg) (filled triangles) on allergen-evoked hyperalgesia in IgE passively sensitized rats. The negative control group consisted of IgE passively sensitized rats challenged with saline (filled circles). Values are means + SEM from at least six animals. $^*P < 0.05$ as compared to saline stimulated rats.

in animals with sustained hyperalgesic response. Results showing that the long lasting hyperalgesic response were impaired by blockade of neutrophil recruitment or systemic neutrophil depletion support the interpretation that components of the cellular immune reaction are crucial to amplify and sustain the immediate IgE-dependent hyperalgesic response triggered by allergen, in a process in which the subsequent recruitment of neutrophils seems to be pivotal.

Since the mechanisms implicated in the processing of nociception vary and are certainly a reflection of the type of stimulus applied (Julius and Basbaum, 2001), our motivation was to dissect the role of IgE-mediated mechanisms and putative participation of leukocytes in the processing of allergen-evoked nociception. In this study, allergen injected into the rat hind paws was the model chosen to assess thermal hyperalgesia in animals subjected to active sensitization in comparison with those passively sensitized by the adoptive transfer of an anti-DNP IgE monoclonal antibody. Our group has reported that local thermal hyperalgesia is indeed a feature of allergen-evoked inflammation and that a synergistic interaction among bradykinin, 5-hydroxytryptamine and histamine is deeply implicated in this phenomenon (Lavich et al., 2003). Nevertheless, allergen-induced inflammation has been shown to be much more complex in actively sensitized animals than in IgE passively sensitized ones, concerning the magnitude of leukocyte infiltration and duration of response (Sorkness et al., 1988, 1990; Lima et al., 1997).

Cutaneous and other forms of allergic responses are marked by early and late phase reaction (Poole and Rosenwasser, 2005). The former, apparent within minutes, is usually associated with a massive release of mast cell derived mediators, including vasoactive amines, lipid mediators and cytokines, and resulting in vascular permeability increase and tissue swelling. The latter, appar-

ent within hours, is characteristically prolonged and followed by persistent swelling and leukocyte recruitment. Active sensitization performed with the help of aluminum hydroxide as adjuvant favors IgE synthesis (Lima et al., 1997). However, there are many reasons to believe that other mechanisms, in addition to those mediated by IgE, are implicated in the evolution from an early to a late phase reaction, and that inflammatory cells are pivotal in the amplification and sustainability of the late phase response. We demonstrated here that both active and IgE passively sensitized rats reacted with a rapid and comparable thermal hyperalgesic response following antigen provocation. Nevertheless, the hyperalgesia is longer-lasting in the actively sensitized group. One putative explanation for these data is that the active sensitization, though effective in stimulating IgE production as reported (Lima et al., 1997), may also have other independent immunologic properties driving the tissue to a state of prolonged hyperalgesia.

Accumulation of neutrophils in the tissue during inflammation is a common event in acute, non-immunological states and in various immunological inflammatory reactions (Sedgwick et al., 1985). In addition, the crucial role of neutrophils in processing pain mechanisms and hyperalgesia has been demonstrated in several inflammatory states, including nerve injury, rheumatoid arthritis and nerve growth factor-evoked inflammation (Bennett et al., 1998; Schuligoi, 1998; Perkins and Tracey, 2000; Bombini et al., 2004). Several studies suggest that the presence of neutrophils in these settings is accounted for by an endogenous release of chemotactic cytokines, like IL-8 and IL-6 (Ordóñez et al., 2000; Alexis et al., 2002; Bochenska-Marciniak et al., 2003; Cunha et al., 2003). Moreover, the involvement of these cytokines in hyperalgesic environments provided by stimuli such as carrageenin, LPS and allergen has also been reported (Ferreira, 1993; Lorenzetti et al., 2002; Cunha et al., 2003). In the present conditions, the number of neutrophils recruited from 180 to 360 min after allergen provocation into paws under passive sensitization appeared increased as revealed by tissue MPO activity values, but this increase was significantly lower compared to that noted following active sensitization. These results are in line with prior studies reporting that, in comparison with actively sensitized rats, IgE passively sensitized animals respond to allergen provocation with mild polymorphonuclear cell mobilization at sites such as pleural cavity (Lima et al., 1997) and lung airways (Sorkness et al., 1988, 1990). These findings support the interpretation that, in addition to the IgE-mediated system, other mechanisms, notably those involving activation of CD4⁺ T cells of the T_H2 phenotype (Caramori et al., 2004), play an important role in up-regulating leukocyte recruitment in allergic conditions.

While trying to define the role of neutrophil infiltration in allergen-evoked hyperalgesia, we decided to deplete the neutrophils by treating actively and IgE passively sensitized rats with a rabbit anti-rat neutrophil antiserum, using a previously reported protocol (Bennett et al., 1998). Our findings revealed that in contrast to what was observed in animals treated with normal serum, those treated with anti-rat neutrophil antiserum failed to sustain the allergen-evoked hyperalgesic response in its late phase. The pattern of installation of the process remained unaltered but there was a marked shortening of hyperalgesia in actively sensitized rats depleted of neutrophils. In line with these findings, we demonstrated the capacity of the selectin inhibitor fucoidin to inhibit neutrophil accumulation as well as the late hyperalgesic response, adding support to the interpretation that neutrophil-mediated mechanisms may indeed be operative in the sustaining phase of the allergic nociceptive process in actively sensitized rats. Thus, neutrophil infiltration seems to influence the duration of allergic thermal hyperalgesia, rather than simply function as an epiphenomenon in this setting.

Of note, MPO levels were still significantly elevated at 360 min after allergen provocation in passively sensitized rats, at a time when hyperalgesia was unapparent in that group. Furthermore, allergen-evoked hyperalgesia in IgE passively sensitized rats remained unaltered following treatment with either anti-neutrophil antiserum or fucoidin. These findings seem to indicate that the neutrophil infiltration might be necessary but not sufficient to cause hyperalgesia, and so an appropriate activation of these cells in the focus of the lesion would appear to be required. Experiments are now underway in order to clarify better this point.

In conclusion, our findings suggest that the IgE-mediated mechanism alone may account for the installation of thermal hyperalgesia, but not for the sustained hyperalgesic response that characterizes the allergic inflammatory reaction observed in actively sensitized rats. Our findings also provide evidence of the crucial involvement of neutrophils in processing the late but not the early phase of the nociceptive process, indicating that allergen-evoked hyperalgesia in actively sensitized rats results from a combination of IgE- and neutrophil-mediated mechanisms.

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References

- Alexis N, Griffith K, Almond M, Peden DB. IL-4 induces IL-6 and signs of allergic-type inflammation in the nasal airways of nonallergic individuals. *Clin Immunol* 2002;104:217–20.
- Bennett G, al-Rashed S, Hoult JR, Brain SD. Nerve growth factor induced hyperalgesia in the rat hind paw is dependent on circulating neutrophils. *Pain* 1998;77:315–22.
- Bochenska-Marciniak M, Kupczyk M, Gorski P, Kuna P. The effect of recombinant interleukin-8 on eosinophils' and neutrophils' migration in vivo and in vitro. *Allergy* 2003;58:795–801.
- Bombini G, Canetti C, Rocha FA, Cunha FQ. Tumour necrosis factor-alpha mediates neutrophil migration to the knee synovial cavity during immune inflammation. *Eur J Pharmacol* 2004;496:197–204.
- Bradley PP, Priebe DA, Christensen RD, Rothstein G. Measurement of cutaneous inflammation: estimation of neutrophil content with an enzyme marker. *J Invest Dermatol* 1982;78:206–9.
- Brown JA. Sinusitis and headache. *J S C Med Assoc* 1991;87:502–4.
- Calheiros AS, Aguiar Pires AL, Pereira da Silva J, Cordeiro RS, Martins MA, Lima MC. Role of the IgE-mediated system in eosinophil recruitment triggered by two consecutive cycles of sensitisation and challenge in rats. *Int Arch Allergy Immunol* 2001;126:325–34.
- Caramori G, Ito K, Adcock IM. Targeting Th2 cells in asthmatic airways. *Curr Drug Targets Inflamm Allergy* 2004;3:243–55.
- Corry DB, Kheradmand F. 7. Control of allergic airway inflammation through immunomodulation. *J Allergy Clin Immunol* 2006;117:S461–4.
- Cunha JM, Sachs D, Canetti CA, Poole S, Ferreira SH, Cunha FQ. The critical role of leukotriene B4 in antigen-induced mechanical hyperalgesia in immunised rats. *Br J Pharmacol* 2003;139:1135–45.
- Ferreira SH. Inflammatory pain: the role of cytokines and its control by drugs which release nitric oxide. *Ann Ist Super Sanita* 1993;29:367–73.
- Fu SY, Gordon T. The cellular and molecular basis of peripheral nerve regeneration. *Mol Neurobiol* 1997;14:67–116.
- Herrera GA. Ultrastructural immunolabeling in diagnostic surgical pathology: illustrative cases. *Ultrastruct Pathol* 1992;16:71–85.
- Heyer GR, Hornstein OP. Recent studies of cutaneous nociception in atopic and non-atopic subjects. *J Dermatol* 1999;26:77–86.
- Julius D, Basbaum AI. Molecular mechanisms of nociception. *Nature* 2001;413:203–10.
- Kaneko M, Schimming A, Gleich GJ, Kita H. Ligation of IgE receptors causes an anaphylactic response and neutrophil infiltration but does not induce eosinophilic inflammation in mice. *J Allergy Clin Immunol* 2000;105:1202–10.
- Kidd BL, Urban LA. Mechanisms of inflammatory pain. *Br J Anaesth* 2001;87:3–11.
- Lavich TR, Cordeiro RS, Calixto JB, e Silva PM, Martins MA. Combined action of vasoactive amines and bradykinin mediates allergen-evoked thermal hyperalgesia in rats. *Eur J Pharmacol* 2003;462:185–92.
- Lavich TR, Cordeiro RS, Silva PM, Martins MA. A novel hot-plate test sensitive to hyperalgesic stimuli and non-opioid analgesics. *Braz J Med Biol Res* 2005;38:445–51.
- Levine JD, Taiwo YO, Collins SD, Tam JK. Noradrenaline hyperalgesia is mediated through interaction with sympathetic postganglionic neurone terminals rather than activation of primary afferent nociceptors. *Nature* 1986;323:158–60.
- Lima MC, Prouvost-Danon A, e Silva PM, Chagas MS, Calheiros AS, Cordeiro RS, et al. Studies on the mechanisms involved in antigen-evoked pleural inflammation in rats: contribution of IgE and complement. *J Leukoc Biol* 1997;61:286–92.
- Lorenzetti BB, Veiga FH, Canetti CA, Poole S, Cunha FQ, Ferreira SH. Cytokine-induced neutrophil chemoattractant 1 (CINC-1) mediates the sympathetic component of inflammatory mechanical hypersensitivity in rats. *Eur Cytokine Netw* 2002;13:456–61.
- Ordóñez CL, Shaughnessy TE, Matthay MA, Fahy JV. Increased neutrophil numbers and IL-8 levels in airway secretions in acute severe asthma: clinical and biologic significance. *Am J Respir Crit Care Med* 2000;161:1185–90.
- Perkins NM, Tracey DJ. Hyperalgesia due to nerve injury: role of neutrophils. *Neuroscience* 2000;101:745–57.
- Poole JA, Rosenwasser LJ. The role of immunoglobulin E and immune inflammation: implications in allergic rhinitis. *Curr Allergy Asthma Rep* 2005;5:252–8.
- Schuligoi R. Effect of colchicine on nerve growth factor-induced leukocyte accumulation and thermal hyperalgesia in the rat. *Naunyn Schmiedeberg's Arch Pharmacol* 1998;358:264–9.
- Sedgwick AD, Moore AR, Al-Duaij AY, Edwards JC, Willoughby DA. The immune response to pertussis in the 6-day air pouch: a model of chronic synovitis. *Br J Exp Pathol* 1985;66:455–64.
- Sorkness R, Blythe S, Lemanske Jr RF. Pulmonary antigen challenge in rats passively sensitized with a monoclonal IgE antibody induces immediate but not late changes in airway mechanics. *Am Rev Respir Dis* 1988;138:1152–6.
- Sorkness R, Johns K, Castleman WL, Lemanske Jr RF. Late pulmonary allergic responses in actively but not passively IgE-sensitized rats. *J Appl Physiol* 1990;69:1012–21.
- Stosh VI, Shugailov IA, Moskovets ON, Shul'gin EG, Myl'nikov AV. Characteristics of the pain sensitivity in patients with accompanying allergic diseases prior to stomatologic surgery. *Anesteziol Reanimatol* 1991:31–4.
- Taiwo YO, Levine JD. Effects of cyclooxygenase products of arachidonic acid metabolism on cutaneous nociceptive threshold in the rat. *Brain Res* 1990;537:372–4.
- Taiwo YO, Levine JD. Serotonin is a directly-acting hyperalgesic agent in the rat. *Neuroscience* 1992;48:485–90.
- Van Arman CG, Nuss GW, Winter CA, Flataker L. Proteolytic enzymes as mediator of pain. In: Lim RKS, Armstrong D, Pardo EG, editors. *Pharmacology of pain*. Oxford: Pergamon; 1968. p. 25–32.
- Williams CM, Galli SJ. The diverse potential effector and immunoregulatory roles of mast cells in allergic disease. *J Allergy Clin Immunol* 2000;105:847–59.